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Microplastic ingestion by an aquatic ciliate: Functional response, modulation, and reduced population growth

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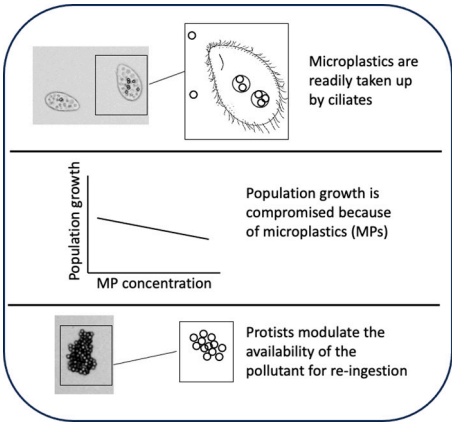
HIGHLIGHTS

- The mechanisms underpinning microplastic uptake by a model freshwater ciliate and consequences for population growth were assessed.
- Microplastic uptake increased in a saturating fashion with concentration.
- Microplastic uptake by ciliates decreased through time, driven by the immobilisation of microplastics after egestion.
- Ciliate population growth was compromised by feeding on microplastics.
- Simple predator-prey models explained the results, providing a basis to understand the fate of microplastic uptake and transfer in food webs.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastic particles are ubiquitous in aquatic environments and are considered a major threat to the large range of heterotrophic organisms that involuntarily consume them. However, there is current uncertainty around the mechanisms underpinning microplastic uptake by aquatic consumers and the consequences for both the fate of the microplastics and the growth potential of consumer populations. We performed a feeding experiment, exposing a model freshwater ciliate, *Tetrahymena pyriformis,* to six different microplastic concentrations and measured microplastic uptake and population growth over the course of several generations. Microplastic uptake increased in a saturating fashion with concentration, consistent with a Type II functional response, with a maximum feeding rate of 22 microplastic particles individual^{-1} h^{-1}. Interestingly, microplastic uptake decreased through time and we observed that, after egestion, microplastic particles aggregated, rendering them too large for re-consumption. We built and tested a simulation model which matched rates of microplastic uptake when incorporating functional response parameters and assuming 50 % immobilisation of microplastics after egestion. Nevertheless, ciliate population growth was compromised by the presence of microplastics, decreasing by 43 %

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over the full microplastic concentration range. Taken together, our results demonstrate the potential for aquatic ciliates to play an important role in the uptake, transfer, and modification of microplastics in freshwater environments with associated negative impacts on population fitness.

1. Introduction

Plastic pollution of aquatic ecosystems is a growing environmental concern (Krause et al., 2021; Kukkola et al., 2021; Rakib et al., 2023). Importantly, much of this pollution is invisible to the naked eye: so-called micro-plastics (particles <5 mm) are ubiquitous in both marine and freshwater environments (Krause et al., 2021; Kukkola et al., 2021). These small particles are consumed incidentally by a vast spectrum of organisms, with associated lethal and sub-lethal effects (Kukkola et al., 2021). Notably, the scope of plastic pollution extends beyond marine environments, where most research has been conducted to date (e.g. Lusher, 2015; Martin et al., 2022), with freshwater systems such as streams and rivers also bearing substantial plastic loadings (Bellasi et al., 2020; Krause et al., 2021; Strokal et al., 2023; Triebskorn et al., 2019). Because of their small size and varying density, microplastics are ever present in the water column and sediments, making them bioavailable for interaction with a range of aquatic organisms, leading to concerns about ecotoxicity and bioaccumulation in food chains (EFSA, 2016). However, the dynamics of microplastic ingestion by aquatic consumers, the implications for population fitness and transfer through the wider food web are poorly understood (Krause et al., 2021; Kukkola et al., 2021).

Most microplastics are similar in size to bacteria, single celled algae, and other microbes (<1 mm) and this makes these pollutants available for incidental ingestion by a wide range of freshwater organisms. Heterotrophic protists, such as many bacterivorous ciliate species, occupy a central position in the microbial loop compartment and therefore represent an important conduit for microplastic uptake into aquatic food webs (Bulannga and Schmidt, 2024). Indeed, microplastics have long been used as 'prey' in protist feeding experiments in the form of microbeads (Boenigk et al., 2001, 2002; Fenchel, 1980a; Lavin et al., 1990; Mueller et al., 1965; Pace and Bailiff, 1987; Ricketts, 1971) because they allow accurate estimates of food intake and calculation of metrics such as clearance rate (Boenigk et al., 2002). These studies have shown that a wide range of heterotrophic protist species can ingest microplastics (e.g. Fenchel, 1980a), that consumption rates are strongly related to particle size (Dubowsky, 1974; Fenchel, 1980a), and that protists tend to not discriminate between microplastics and suitable prey even though plastics per se have no nutritious value for them (Dubowsky, 1974; Fenchel, 1980a). Although some recent research on microplastic pollution has harnessed this knowledge (Bulannga and Schmidt, 2022, 2024; Nałęcz-Jawecki et al., 2021; Nugroho and Fyda, 2020) there is a need to place microplastic consumption by heterotrophic protists within a conceptual framework that goes beyond identifying which protists ingest microplastic (Rillig and Bonkowski, 2018) to providing parameters that can be scaled up to simple ecosystem models (e.g. such as those based on feeding rates applied for carbon flow in biofilms [Weitere et al., 2018]).

In this vein, functional response models are a valuable tool for parameterising an organism's feeding behaviour - i.e. how feeding rate changes with varying levels of resource availability. These models have been central in understanding trophic interaction strengths, population dynamics and ecosystem stability (Berlow et al., 2009). Recently, this approach has been used in microplastic research to determine microplastic ingestion at a range of concentrations (e.g. for fishes; Mbedzi et al., 2020), as well as the indirect effects of microplastic exposure on predator feeding rates (e.g. for shore crabs; Cunningham et al., 2021). In a functional response framework, the feeding rate of a predator depends on the density of its resource, the attack rate, and handling time (Holling, 1959), resulting in three common patterns (Type I, II and III). A Type I functional response is characterized by a linear increase in feeding rate with prey density. In contrast, a Type II functional response is characterized by a response curve with high feeding rates at low prey densities and a saturation at high prey densities. This feeding behaviour is a consequence of a significant handling time, which does not exist in Type I functional response (Holling, 1959). In a context where consumers feed on microplastic 'prey', a Type II functional response would be indicative of significant handling time (i.e., the time needed to attack, consume, and digest the prey) yet high consumption rates, even under low environmental concentrations (Mbedzi et al., 2020). In contrast, a Type III functional response, characterized by a sigmoidal model, would signify an accelerating attack rate at low prey densities (as at intermediate prey densities this feeding becomes directly density-dependent and eventually levels off at high prey densities). The latter relationship could be driven by patchily distributed microplastic particles and predators actively foraging on these patches (Holling, 1966; Oaten and Murdoch, 1975).

Whether the uptake of microplastics by ciliates follows one of these functional response types is unknown and various factors could complicate the search for such general patterns. First, some heterotrophic protists can, under certain conditions (e.g. with starvation and low food concentration), discriminate between microplastics and microbial prey (Boenigk et al., 2001, 2002; Dubowsky, 1974; Ricketts, 1971) meaning that microplastics could be avoided, even in the absence of other legitimate prey. Second, ciliates egest inert particles and organic matter while feeding meaning that microplastic particles can be 'consumed' multiple times, unlike true prey. Finally, although protists do not break microplastics down while feeding (unlike larger invertebrates [Mateos-Cárdenas et al., 2020]), egested particles and organic matter can often form aggregates (Parry et al., 2001) potentially reducing the bioavailability for (re)ingestion and affecting uptake rates in the long term.

In addition to the need for describing the basic mechanisms of microplastic uptake, little is known about if and how feeding on microplastics compromises the growth of heterotrophic protist populations (but see Bulannga and Schmidt, 2022; Nugroho and Fyda, 2020; Wu et al., 2021). Because of their small size, protists have a high feeding rate and reproduce rapidly (Fenchel, 1974; Reiss and Schmid-Araya, 2010). It is conceivable that population growth will decline for consumer populations exposed to increasing microplastic concentrations (Wu et al., 2021), since the consumption of these provides no nutritious value (Parry et al., 2001), and the formation of vacuoles during feeding comes at a significant energetic cost (Skriver and Nilsson, 1978).

Here we performed a controlled feeding experiment to assess rates of microplastic ingestion by a model aquatic heterotrophic protist, *Tetrahymena pyriformis*, at a range of microplastic concentrations and estimated the impacts on population growth. Our overall aim was to develop and test a simple simulation model of *T. pyriformis* feeding through time.

We hypothesised that:

1. Microplastic consumption by the ciliate follows a Type II functional response relationship with a maximum feeding rate determined by 'handling' time.
2. Microplastic load in ciliates decreases through time as particles aggregate after egestion into faecal pellets rendering them less available for ingestion.
3. Population growth decreases with increasing microplastic concentrations as ingestion of non-nutritious particles increases energy expenditure.

2. Methods

2.1. Model consumer: T. pyriformis

A commercial culture of the ciliate *T. pyriformis* (CCAP1630/1W) was obtained from the Culture Collection of Algae and Protozoa (CCAP, SAMS Limited, Scottish Marine Institute, Scotland, United Kingdom) and sub-cultured in axenic conditions until the feeding experiment commenced. This species was selected as a model consumer due its presence across various freshwater habitats and its ability to be cultured under axenic conditions (i.e. without bacterial prey [Sauvant et al., 1999]) ingesting both inert (e.g. latex beads) and digestible artificial particles when they are of comparable size to bacteria (Dürichen et al., 2016; Lavin et al., 1990).

Subculturing was performed in a Class II Biological Safety Cabinet (BSC) under sterile conditions. This involved pipetting 0.5 ml of culture into 50 ml of Proteose Peptone yeast extract medium containing 20 g proteose peptone and 2.5 g yeast extract l^{-1} (Fussmann et al., 2014) into autoclaved (100 ml) Erlenmeyer glass flasks with metal caps (Altermatt et al., 2015). Cultures were maintained at 20 °C in a thermostatically controlled cabinet (Lovibond, TC255S, Germany) to ensure rapid growth of the population and were regularly observed under the microscope to check for contamination and monitor population growth. Sub-culturing took place every 3–4 days to ensure populations were maintained in the exponential growth phase and in replete resource conditions.

2.2. Microplastic particles

Polystyrene microspheres (Phosphorex Inc., UK) of two different sizes were used as microplastics in the feeding experiment. 4 μm beads (Phosphorex™ 2106) were used as 'prey' for *T. pyriformis* after performing initial feeding trials with different size particles and consulting the literature (Dürichen et al., 2016; Jost et al., 1973; Lavin et al., 1990; Mueller et al., 1965; Nilsson, 1977). 10 μm microplastic beads (Phosphorex™ 2106G) were found not to be consumed by *T. pyriformis* (as also described by Bulannga and Schmidt, 2022) and hence were used as a 'control' in the experiment. The microplastic particles were washed twice with deionised water and centrifuged to ensure the removal of the product solution (2 mM NaN_3). The washing was performed by centrifuging the 1 ml tubes containing the suspended microparticles at 5000 *g* for 15 min and by discarding the supernatant before topping up with deionised water (after Horton et al., 2018).

2.3. Feeding experiment

The feeding experiment ran for 24 h and was performed in 2 ml microcosms (glass screw cap tubes) at 20 °C. A dilution series of the 4 μm microplastic particles was produced by adding deionised water and a small amount of the original suspension to give six different initial concentrations ranging from 15×10^6 to 7500 particles ml^{-1}. Each microcosm received 650 μl of axenic culture medium with *T. pyriformis* and 100 μl of one of the six concentrations of 4 μm microplastics (750 μl in total), previously vortexed using a Whirlimixer (Fisons, UK) to ensure an even distribution of particles. Consequently, six different final microparticle concentrations were established: 1000; 10,000; 100,000; 500,000; 1×10^6 and 2×10^6 particles ml^{-1}. In addition, a 'control' was established by adding 100 μl deionised water containing 10 μm microplastic particles (too large for *T. pyriformis* ingestion), giving a final concentration of 16.5×10^6 particles ml^{-1}. No other feeding source (other than nutrient in the medium) was provided, so the feeding behaviour of *T. pyriformis* on microplastics could be explored without the interference of different available resources. A constant density of ciliates of c. 10,000 ciliates ml^{-1} was achieved in each microcosm by diluting the culture with culture medium prior to the start of the experiment (resulting in an exposure range of 0.1 to 200 microplastic particles $ciliate^{-1}$). Ciliate density was determined by pipetting repeated 10 μl of sample into counting chambers (FastRead BVS100, Immune Systems Ltd., UK) and counting individuals under the microscope (Olympus BX50) at 100× magnification (10× objective). Each of six 4 μm microplastic concentrations and the two controls treatments were replicated four times (except for the lowest concentration where only three replicates were available due to lost data). The experiment was performed at 20 °C with microcosms constantly mixed on a tube roller to keep the microplastics in suspension. We observed the number of microplastic particles ingested per predator at nine time points, bringing the total number of observations up to 243 ([5 concentration treatments *4 + low concentration treatment *3 + microplastic control *4] * 9). In addition, population growth was estimated at the end of the experiment in all microcosms (described below).

Over the 24 h period, one sample of 15 μl was taken out of the microcosm at nine time points (2, 10, 20, 30, 45, 60, 120, 240 and 1440 min) to observe ciliates under the microscope, with their movement slowed down by one drop of 'protoslow' - Methyl cellulose (Sigma-Aldrich, UK). Ingested microplastics were counted per ciliate by recording a short video (of up to four minutes) under an Olympus BX50 microscope fitted with an Infinity 3 camera (Lumenera, Canada). Videos were analysed by manually counting the number of ciliates in the frame and the number of ingested microplastic particles per cell. Ingested microplastic particles were clearly visible within the cell vacuoles (Fig. S1). At the time point of 1440 min (24 h), three samples of 15 μl were additionally examined in a counting chamber to analyse the growth rate of *T. pyriformis*.

2.4. Data analysis

The rate of microplastic particle ingestion as a function of microplastic concentration (i.e. the feeding functional response) was quantified using data on the number of particles consumed by ciliate populations after 20 min of microplastic exposure. This timeframe was chosen to ensure microplastic concentrations in the environment remained non-limiting and egestion had not yet occurred. For example, based on feeding studies with *Tetrahymena* spp. and *E. coli* (Jost et al., 1973) we might expect simple functional response mechanisms to apply (Holling, 1959), at least during the initial feeding phase when microplastics are readily available and have not aggregated due to faecal pellets.

In the functional response framework, the feeding rate, F, of a predator, depends on the density of its resource, S, the attack rate, a, and handling time h:

$$F = \frac{aS^{(q+1)}}{1 + ahS^{(q+1)}}$$

The function describes a functional response of Type II (when $q = 0$) or Type III (when $q = 1$) (Pritchard et al., 2017). Functional response fitting was performed using the 'frair' package (Pritchard et al., 2017) in R statistical software (RCore Team, 2022). First, data were visualized, and model selection was performed using the frair_test function to identify the form of the functional response (Type I, II or III). In all three types of functional response described by Holling (1959) a constant availability of prey is assumed. However, this is often not the case in experimental studies, or in nature. Since resources (microplastic particles) were not replenished (i.e. once some of the prey has been eaten, the available density is lower than at the beginning of the experiment) 'rogersII' models were applied for fitting a suitable functional response model for the obtained data (Pritchard et al., 2017; Rogers, 1972). This approach does not account for potential predator interference (Fussmann et al., 2014). Second, we obtained, a, the attack rate (ml / ind / min), and h, handling time (min), from the final model.

To better understand the feeding dynamics, we used the values of a and h obtained from the functional response fitting to parametrize a differential equation model of *T. pyriformis* feeding through time

simulated in R with the deSolve package. In the model, microplastic particles can be suspended in the medium S, or ingested by *T. pyriformis* I. After a certain digestion time τ, ingested particles are eventually released from the vacuoles of *T. pyriformis*, they leave the ingested pool, and they can either go back to the suspended pool S, with a certain probability p, or they can move to a different pool of rejected particles R that are no longer available for re-ingestion by *T. pyriformis*. The model equations are as follows:

$$S' = -\frac{aS}{1+ahS}T + \frac{1}{\tau}pI$$

$$I' = \frac{aS}{1+ahS}T - \frac{1}{\tau}I$$

$$R' = \frac{1}{\tau}(1-p)I$$

$$T' = rT$$

where T is the density of *T. pyriformis* and T' models its population growth with an exponential growth rate r.

The model allows us to qualitatively explore the impact of the digestion time τ and of the probability of re-ingestion p on the uptake of particles. In fact, if at the beginning of the experiment the number of particles visible inside the vacuoles of *T. pyriformis* essentially increases with feeding $I' = \frac{aS}{1+ahS}T$, then, in the longer term this number is determined by a balance between ingestion and rejection $\left(-\frac{1}{\tau}I\right)$, where ingestion is also affected by the availability of particles in the S pool. Parameters a and h were fitted directly from the data, parameter τ was set to $\tau = 90 / \log(2)$ min (corresponding to a half-life of vacuoles of 90 min, to approximately match values reported in the literature e.g. Nilsson, 1977), r was set to $r = 2\log(2) / 24 / 60\ \text{min}^{-1}$ (a growth rate of c. 2 generations per day, observed for *T. pyriformis* under 'control' conditions at the experimental temperature). Parameter p is only explored qualitatively (e.g. if $p = 100\%$ all rejected particles are available for consumption again, while for $p < 100\%$ a fraction of particles become unavailable) because its exact value would mainly affect the temporal distribution of ingested particles at time scales for which we have few data available; in the figures presented in this manuscript $p = 50$ %.

Finally, to explore if microplastic exposure had a negative impact on *T. pyriformis* growth rate, population growth was calculated after 24 h. Assuming exponential growth throughout the experiment, the growth rate (generations day^{-1}) was calculated as $\log_2(N(t)/N(0))/d$ where $N(0)$ is the density of ciliates at the beginning of a culture, $N(t)$ is the final density of ciliates in the culture and d is the length of the culturing period in number of days. A linear regression was performed between growth rate and microplastic concentration using R statistical software (RCore Team, 2022). The growth rate of *T. pyriformis* exposed to 10 μm particles (too large to be ingested) was included in the analysis (i.e. zero 4 μm microplastic concentration). Microplastic concentrations were log +1 transformed to meet assumptions of the model.

3. Results

3.1. Functional response

Microplastic particle ingestion increased as a function of microplastic concentration over the first 20 min of the experiment, in a saturating fashion, characteristic of a Type II functional response

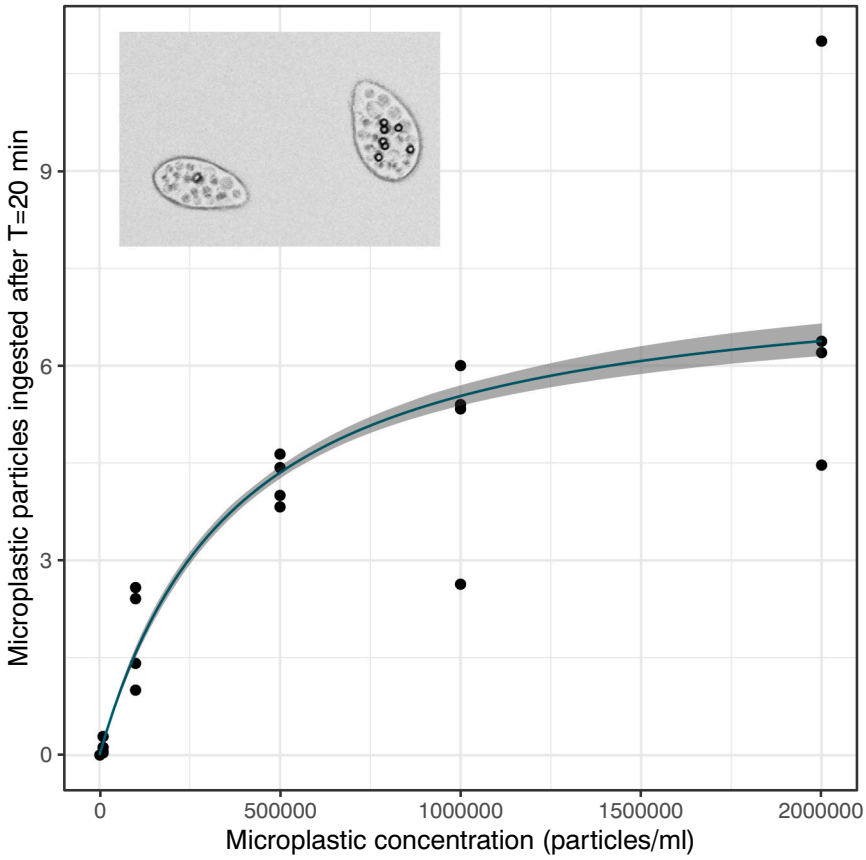

Fig. 1. Functional response of the ciliate *Tetrahymena pyriformis* feeding on microplastic particles. The shaded band represents the 95 % confidence interval of the model fit. Ingestion values are per ciliate and are calculated from data recorded during the first 20 min of the experiment. The photo insert shows two individuals with ingested microplastic particles in food vacuoles.

(Fig. 1). Model selection revealed very strong evidence for a Type II functional response (z-value = − 2519.3, p-value <0.001). The resulting fitted model estimated an attack rate of 1.08×10^{-6} ml / minute ($\pm$ 7.17×10^{-10}) and an average handling time of 2.67 min ($\pm$ 9.1×10^{-4}). The inverse of the handling time corresponds to the maximum feeding rate, F_{max}, which was estimated to be 0.37 microplastic particles per minute - i.e. 22.2 particles individual^{-1} h^{-1}.

3.2. Simulation model of decreased feeding over time

Over the course of 24 h the mean number of ingested microplastic particles per *T. pyriformis* individual varied notably (Fig. 2). Initially, the amount of ingested microplastic particles increased sharply over time, but this trend slowed within the first four hours of the experiment. After 24 h, the number of ingested microplastic particles per ciliate was lower

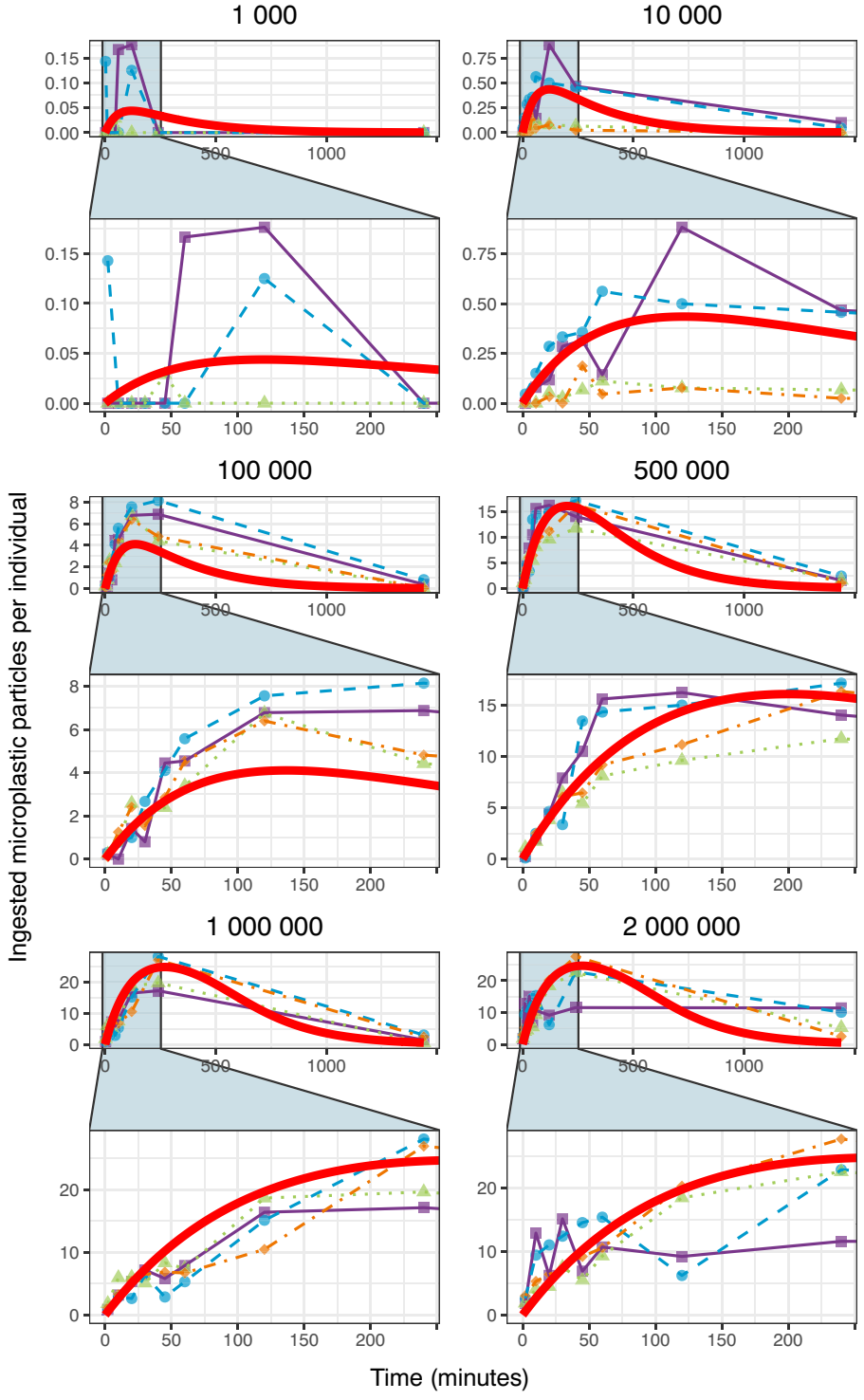

Fig. 2. Mean per capita ingestion of microplastic particles by ciliate *Tetrahymena pyriformis* over 24 h at different microplastic concentrations. A zoom on the first 4 h of the experiment is shown for clarity, creating two panels for each concentration. The solid red line represents the simulation model, which incorporates the functional response parameters (calculated from particle ingestion over the first 20 mins, see Fig. 1) and assumes 50 % of the particles become available for re-ingestion after being released from the vacuoles. The different colour lines and symbols indicate different replicates in the experiment.

than that observed after 4 h. This was consistent for each microplastic concentration treatment (Fig. 2).

The simulation model, which incorporates the functional response parameters and assumes 50 % of the microplastic particles become unavailable after ingestion, provided a reasonable fit to the data (Fig. 2). If particles were fully available for re-ingestion after being released from the vacuoles, all curves should monotonically increase over time (assuming no population growth). Aggregates of microplastic particles were frequently overserved in the samples (Appendix Fig. S1b), especially at later time points, and were observed to be unavailable for (re) consumption (Appendix Video S3). This decreasing pattern in consumption over 24 h holds even after we accounted for the growth of *T. pyriformis* during the experiment (Appendix Fig. S2).

3.3. Effects of microplastic ingestion on growth rate

There was strong evidence that the growth rate of *T. pyriformis* (over 24 h) decreased with increasing microplastic concentration (Fig. 3; $F_{(1,25)} = 17.03$, p-value <0.001). The intercept of the model (i.e. growth rate in the absence of 4 μm microparticles) was 2.5 ± 0.20 generations day^{-1}. The average growth rate decreased by 43.2 % over the full microplastic concentration range with a growth rate of 1.42 generations day^{-1} estimated by the model at the highest microplastic particle concentration (2×10^6 particles ml^{-1}; 200 particles ciliate^{-1}). Overall, microplastic concentration explained over a third of variation in ciliate growth rate ($R^2 = 0.38$).

4. Discussion

Our study explored the potential for an aquatic ciliate to ingest microplastics across a gradient of concentrations. The uptake of microplastics by *T. pyriformis* was strongly dependent on microplastic concentration and was characterized by a functional response comparable to the uptake of bacterial prey (Sauvant et al., 1999). Interestingly, microplastic ingestion decreased over the course of the experiment. A simple simulation model (we developed around the observation that egested particles tended to form aggregates, that were then too large to be re-consumed) captured the observed decrease in microplastic ingestion dynamics. Still, ciliate population growth was significantly compromised with increasing environmental concentrations of microplastics. Given the high abundance of heterotrophic protists in freshwater environments (Reiss, 2018), especially in polluted systems and sediments (Reiss and Schmid-Araya, 2008, 2010) with high microplastic loadings (Bellasi et al., 2020; Krause et al., 2021; Triebskorn et al., 2019), these patterns likely have significant implications for a range of freshwater systems. Below, we explain these new insights into the dynamics of microplastic uptake and impact on ciliate populations, and their potential implications in natural systems.

4.1. Ingestion of microplastics across concentrations

The ingestion of microplastic particles by the ciliate exhibited a positive but saturating relationship with increasing concentrations. We found the pattern to align with a Type II functional response (Holling, 1959), where proportional uptake rates by predators are highest under low prey densities (i.e., microplastic concentrations). It follows that *T. pyriformis* actively uptakes microplastics even when those are relatively rare in the environment, but significant handling time limits a further increase in the ingestion rate of microplastics. From the functional response model, we were able to estimate the average handling time to be ~2.67 min per microplastic particle, which is comparable to feeding rates on natural food particles by *Tetrahymena* (Nilsson, 1977). The inverse of the handling time corresponds to the maximum feeding rate, F_{max}, providing key quantitative insights into the potential for this species to uptake microplastics from the environment. In our experiment this was 22 particles individual^{-1} h^{-1}. It is important to note that our experimental units contained more than one 'predator' individual, which could potentially lead to an increase in predator interference,

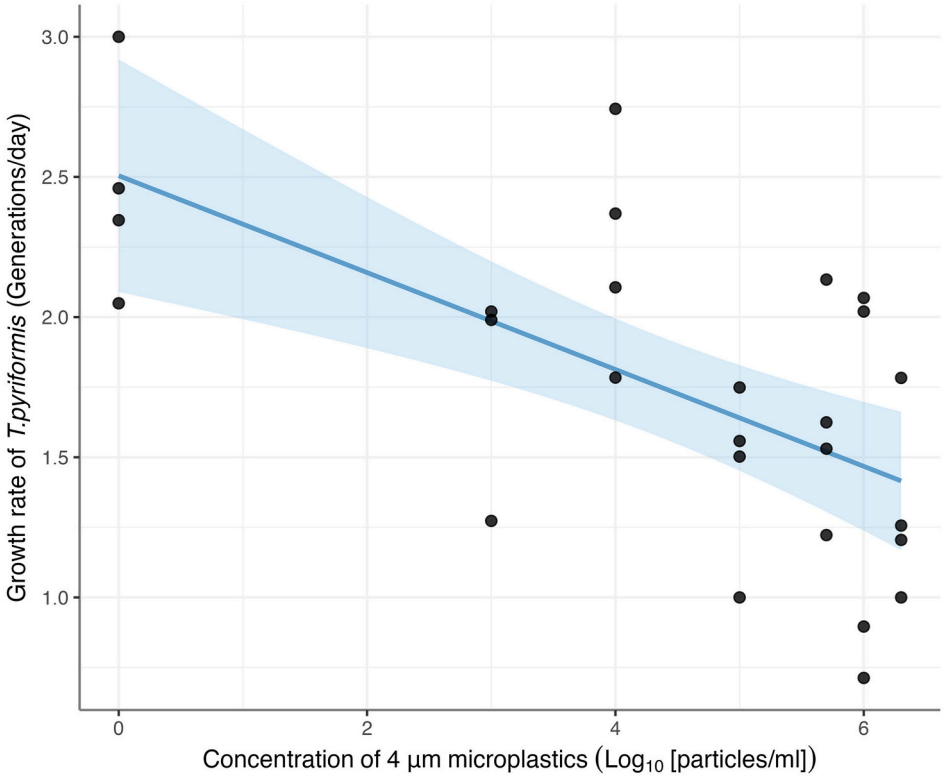

Fig. 3. Growth rate of *Tetrahymena pyriformis* as a function of microplastic particle concentration. The regression fit is shown including the 95 % confidence interval. *T. pyriformis* exposed to 10 μm particles (too large to be ingested) is used for microplastic concentration = 0. Microplastic concentrations are $\log_{10} + 1$ transformed.

reducing maximum feeding rates (Fussmann et al., 2014). Nonetheless, this result is not surprising given that *Tetrahymena* sp. has been reported to graze on 3×10^3 bacteria ciliate^{-1} h^{-1} (Bulannga and Schmidt, 2022) and to clear 5×10^{-5} ml hour^{-1} (Fenchel, 1980b). Given that ciliates can reach abundances of 1 Mio individuals m^{-2} in some (eutrophic) environments (Reiss and Schmid-Araya, 2008), and are consumers of a wide range of microscopic prey, especially in aquatic biofilms (Weitere et al., 2018), there is therefore scope for these organisms to play an important role in the stability and mobility of microplastics in freshwater environments, as recently highlighted by Bulannga and Schmidt (2024). Indeed, different protist groups have been observed to ingest microplastics under a range of environmental conditions, including ciliates (Bulannga and Schmidt, 2022; Fenchel, 1980a; Lavin et al., 1990; Mueller et al., 1965; Nugroho and Fyda, 2020; Pace and Bailiff, 1987; Ricketts, 1971), flagellates (Boenigk et al., 2002; Dubowsky, 1974), and amoebae (Avery et al., 1995; Elloway et al., 2006; Korn and Weisman, 1967; Stewart and Weisman, 1972; Weisman and Korn, 1967). When it comes to differences in microplastic uptake among taxonomic groups or across environmental contexts (e.g. nutrient conditions, temperature etc.), a functional response framework provides a standardised means to better understand key constraints underpinning the uptake of microplastics in natural systems.

4.2. Microplastic modulation and feeding simulation model

We observed that aggregates of microplastic particles tended to form during the later stages of the experiment, and these appeared to be unavailable for (re)ingestion. This points to the potential for modulation of plastic pollution by protists within the microbial food web – a mechanism known for micro-crustaceans such as copepods that egest microplastics in faecal pellets (Cole et al., 2013; Coppock et al., 2019). In ciliates, microplastics are taken up into food vacuoles that have a lower pH than the protist cell and are exposed to proteins such as lysosomes (Nilsson, 1977; Parry et al., 2001). Indeed, many ciliates will egest inert particles and organic matter as faecal pellets that can then form aggregates - as described in detail for *Tetrahymena pyriformis* (Parry et al., 2001) potentially making them unavailable for (re)consumption resulting in reduced feeding rates in the long term.

This likely explains why the average number of microplastic particles ingested by *T. pyriformis* decreased by the end of the experiment (after 24 h of exposure). This decreasing pattern in microplastic ingestion holds even after we accounted for the growth of *T. pyriformis* during the experiment, indicating that it was not driven by the decreasing ratio of available microplastic particles for ciliates (due to population growth). We found that using a simple simulation model, which incorporates the attack rate and handling time parameters from the functional response model and assumes that 50 % of particles become unavailable after consumption provided a reasonable fit to the data. We are unable to provide a more exact estimate of the proportion of 'unavailable' particles as we were unable to measure continuously over the 24-h period due to logistical constraints. Nonetheless, our monitoring through the experiment showed that microplastic ingestion by ciliates decreased through time, concomitant with the reduced availability of microplastics following egestion.

4.3. Microplastics effect on growth rate

As predicted, the growth rate of *T. pyriformis* decreased with increasing microplastic concentration, suggesting that the ingestion of microplastic has a negative effect on reproduction rates and fitness. Similarly, Wu et al. (2021) showed that *T. thermophila* growth was inhibited when exposed to polystyrene nanoplastics. It should be noted that the literature highlights both negative and positive effects of particle presence in the medium – for example, particulate material in the culture has been shown to facilitate vacuole formation which in turn leads to faster population growth (Rasmussen and Kludt, 1970).

However, it is not only intuitive but also conceivable that investing in food uptake without nutritional value will lead to slower growth. In our experiment, we can assume that *Tetrahymena* was severely compromised by forming food vacuoles for inorganic particles (Skriver and Nilsson, 1978). If the individuals had fed on the same amount, and size, of bacteria as they did on microplastics, they would have consumed 70 % of their own carbon body weight within one reproductive cycle, i.e. this would have represented a significant addition to the nutrients that are taken up by pinocytosis (direct uptake of small molecules, which does not involve the oral groove) in this species. These back-of-the-envelope estimates are based on the following assumptions: the ciliate (60 μm long; ellipsoid volume of 0.012 nL, equivalent to 1.3×10^{-6} mg C) completes one generation in twelve hours (i.e. doubling of biomass), feeds on 22 items per hour, prey is 4 μm long (sphere volume of 0.00003 nL, 3×10^{-9} mg C); prey and predator have the same carbon content per unit body mass; and metabolic needs follow known body mass scaling laws (see equations in Reiss and Schmid-Araya, 2010). With a known digestive cycle of 2 h at high temperatures (like in our experiment), and limits on how many food vacuoles can be formed (Nilsson, 1977), microplastics will affect the growth of *Tetrahymena*. These estimates (where microplastic handling comes at an energetic cost) will be more extreme for most heterotrophic protists that cannot feed on nutrients via pinocytosis alone and where microplastics compete with nutrient uptake via phagocytosis to a large extent (Fenchel, 1980a, 1980b).

4.4. Extrapolating to natural systems and outlook

Our experiment, which looked at just part of the complex processes that operate in natural systems, has some notable limitations. For instance, we only used a single type of microplastic particle in this study whereas a spectrum of microplastic sizes, shapes and polymer types can be found in natural systems (Krause et al., 2021). These variables can impact feeding rates and the growth rate of consumer populations. For example, the maximum ingestion rate we observed was 22 particles individual^{-1} h^{-1}. Parry et al. (2001) found a substantially higher ingestion rate of 207 ± 32 particles ciliate^{-1} h^{-1} for *T. pyriformis*, with microparticles that were about 1/8 smaller in diameter compared to the particles used in our experiment. Furthermore, microbial biofilms or layers containing pollutants can develop on microplastic particles and probably alter the effect or rate of their ingestion for larger predators (e.g. Fabra et al., 2021). Prey diversity (differently sized prey and large or inedible 'non-prey') can substantially reduce a predators' feeding rate. These changes to consumers' functional responses in more realistic settings has important implications for consumer-resource dynamics (Kratina et al., 2007; Hammill et al., 2015). In nature, protozoans feed on bacteria (Norf et al., 2009), natural, inert particles (Boenigk and Novarino, 2004) in addition to microplastic pollution (Bulannga and Schmidt, 2024). Importantly, these potential differences in uptake rates of microplastics differing in size, shape, polymer types and age, while other prey items are present, could be examined using a functional response approach. This framework offers a general, standardised approach to better-link laboratory exposure studies to real-world concentrations of microplastics in natural systems (Cunningham et al., 2021; Cuthbert et al., 2019; Mbedzi et al., 2020).

Whilst there is an increasing body of literature documenting microplastic pollution in fresh waters (Bellasi et al., 2020; Krause et al., 2021; Strokal et al., 2023; Triebskorn et al., 2019), to our knowledge, no studies have reported the abundance of small microplastics (e.g. 1–10 μm) in freshwater sediments or open water. Essentially, the 'bacteria-sized' microplastics particles are too small to be considered in studies that filter water over sieves but too large to be considered in studies that address nanoplastic pollution. In freshwater environments, such as rivers, most studies have shown that smaller microplastic particles are far more abundant than larger ones (Tibbetts et al., 2018; and references therein) and that most of the pollution is buried in the sediment (Krause et al., 2021). Yet, the smallest sizes reported, with abundance estimates,

are for particles larger than 63 μm (Tibbetts et al., 2018; and references therein). Based on data from Tibbetts et al. (2018), microplastics can be detected in even a gram of sediment, which corresponds to the low microplastic concentration used in our experiment. There is clearly a need for more data on 'bacteria-sized' microplastic pollution, and future studies should focus on both the smallest particle size range and the uptake of these particles by microscopic consumers, while also including natural, inert, particles (e.g. clay) for comparison (Boenigk and Novarino, 2004).

In natural systems, we can assume that a substantial number of nano- and microplastics are in a constant flux within the microbial food web and can be transferred to higher trophic levels. For instance, ciliates are preyed upon by a wide spectrum of organisms, from copepods (Reiss and Schmid-Araya, 2011) to fish larvae (da Silva et al., 2022), leading to the indirect uptake of microplastics by larger consumers (Athey et al., 2020; Stienbarger et al., 2021). Ciliates can hence be an important conduit for transferring microplastics present in the water body to larger organisms in the aquatic food web (Carbery et al., 2018; Saeedi, 2024; Setälä et al., 2014). Furthermore, the fact that microplastic particles can agglomerate after ciliate egestion could also change microplastic movement in the water column or sediment and ingestion by larger aquatic consumers.

Our study highlights the potential for aquatic ciliates to play an important role in the uptake and modification of microplastics in freshwater environments with associated negative impacts on population fitness. Microplastic ingestion by ciliates is a highly dynamic process, yet simple models grounded in predator-prey theory revealed that two key constraints, handling time and rate of microplastic aggregation after egestion, explained the uptake of microplastics by ciliate populations. These new insights provide a basis to better understand the fate of microplastic uptake and transfer through freshwater food webs.

CRediT authorship contribution statement

Daniel M. Perkins: Writing – review & editing, Supervision, Formal analysis, Conceptualization. **Hedda L. Müller:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Susanne Grünewald:** Data curation. **Julia Reiss:** Writing – review & editing, Supervision. **Katherin Restrepo-Sulez:** Data curation. **Anne Robertson:** Writing – review & editing, Funding acquisition. **Andrea Perna:** Writing – review & editing, Supervision, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.178272.

Data availability

The data underpinning this publication can be accessed from Brunel University of London's data repository, Brunelfigshare under a CCBY licence: https://doi.org/10.17633/rd.brunel.27960867.

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